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EXPERIMENTAL RESEARCHES ON SPECIFIC THERAPEUTICS.

BY

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LECTURE III.

Chemo-therapeutic Studies on Trypanosomes.

The studies on trypanosomes, which have of late been carried out with the greatest energy, especially in the Institut Pasteur in Paris, the School of Tropical Medicine in Liverpool, and the Speyerhaus in Frankfort-on-the-Main, are of the greatest theoretical and practical importance; for in this field of study the patient searching work of Experimental Therapeutics has first come into full activity, and at the same time a basis has been gained on which we may hope successfully to undertake the practical attempt of suppressing sleeping-sickness. The exceedingly great difficulty of these studies is evidenced by the fact that hundreds and thousands of substances have to be examined by animal experiments, before a few producing a therapeutic effect can be found. I myself have in course of time examined more than 600 such substances. From their effect on animals, hints may be obtained as to what to avoid, and in what direction therapeutics should advance. I will not enter here into the details of my work, but will only give you a general *résumé* of the chief results.

The substances which have hitherto proved efficient in combating trypanosome infections can be divided into three groups, viz. :— (1) The group of the basic triphenyl-methane dyes; (2) The group of the benzidine dyes; (3) The group of the arsenicals.

The salts of mercury may be used as adjuvantia to each of the foregoing.

Among the triphenyl-methane stains, which were first employed by Wendelstadt, para-fuchsin is by far the most satisfactory. It is possible, by prophylactic feeding with this drug, to render mice resistant to trypanosome infections for a long time, and I believe

that this innocent substance might well be employed in the Tropics for the prophylaxis of human sleeping-sickness.

As regards the second type of substances, belonging to the benzidine group, trypan-red is a cotton dye which shows the remarkable property of staining mice an intense red, which remains fast for months. I have also prepared a number of similar dyes belonging to the benzidine series, some of which possess an even more powerful action than trypan-red. In several cases a blue dye belonging to the same group, which was prepared by Nicolle and Mesnil, has proved still more efficient.

The value of arsenic acid which has for a long time been employed in the treatment of trypanosome diseases was first recognised by Laveran. Later on it was discovered, at the Liverpool School of Tropical Medicine, that atoxyl was a far more powerful substance. It is prepared by treating aniline with arsenic acid. The makers believe it to be an anilide of arsenic acid, *i.e.*, a compound in which the arsenic acid radical is supposed to be attached to the amido group in a similar manner as the acetic acid is present in acetanilide. Now, as you all know, the ammonia radical, which in aniline is very ready to react, has completely lost this property in acetanilide. This latter body is in fact almost an indifferent compound. Similarly, by the view that atoxyl was the anilide of arsenic acid, any further development of this compound was rendered impossible. I was, however, able to prove, together with Dr. Bertheim, that this compound is to be regarded as something entirely different, namely, that the amido group is quite free and that the arsenic acid radical is attached to the benzol ring in the para position. Atoxyl must, therefore, be regarded as the sodium salt of paramido-phenyl-arsenic acid.

We have obtained a great number of substances derived from it, and have tested them therapeutically. Thus we found that, *e.g.*, by the introduction of the acetyl radical into the amido group the acetyl-amido-phenyl-arsenic acid is produced, which is far less toxic for mice than atoxyl. By means of this substance it is even possible to solve one of the most difficult therapeutic problems imaginable. For mice that have been infected with our most virulent strains, and which, without treatment, would die within three days, can in two-thirds of the cases be saved by this substance if it is given twelve to fourteen hours before death. Such curative results have never yet been published; for in previous communica-

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tions there were mentioned almost exclusively experiments carried out at earlier periods of infection. In our cases acetyl-atoxyl will cure mice in nearly 100 per cent. Unfortunately, acetyl-atoxyl is decomposed in other animals, especially in the horse, into acetic acid and aniline, and it cannot, therefore, be nearly as effective as it is in the mouse. Nevertheless, the results obtained in the mouse are so excellent that they must encourage us to energetically pursue the path of these discoveries.

I mentioned in my last lecture that it is possible to obtain strains of trypanosomes which are resistant to these active substances. Studies, which I have carried out with Dr. Röhl and Dr. Browning, have shown this resistance to be of a very high degree. Thus, atoxyl-fast strains show no sign of being influenced by the highest doses of atoxyl which are applicable to mice. I gave you the explanation for this resistance, viz., that it is the result of a decrease in the avidity of the trypanosomes for these trypanocidal substances. Further, I pointed out that, apart from this resistance, the parasites' protoplasm, though not their nuclei, had become hyper-sensitive towards the arsenical preparation. Thus we meet with an important instance of association, in one and the same cell, of resistance and hyper-sensitivity. This association, which is of late years proving to be of increasing importance in the question of mammalian immunity, is therefore present even in so simple an organism as that of the protozoon.

Although in the mouse atoxyl-resistance usually develops only after a fairly long period, I have occasionally seen it after a fortnight. This observation is very important, because it makes it possible that the negative results observed by Ayres Kopke, Broden, Rodhain, Todd and van Campenhout, in cases of sleeping-sickness which had received a long-continued treatment, may also be due to such a resistance.

In the same way as with atoxyl, trypanosomes can also be rendered resistant to para-fuchsin, trypan-red and trypan-blue, and further, it was shown in my Institute by Franke in his work with apes, that it is even possible for trypanosomes to become resistant to those protective substances which are formed after the recovery of the host from a trypanosome infection. A similar discovery has recently been made by Levaditi in the spirochætes of relapsing fever. This is therefore a general law, and it is especially important, because this change in the trypanosomes, having once become

acquired, remains an hereditary property. Thus I have cultivated for 125 generations my atoxyl-fast strain, without finding any decrease in its powers of resistance. We must further note that every such resistance is specific. Thus the fuchsin-fast strains are resistant only to this substance, but not to atoxyl and trypan-red, and *vice versâ*. This resistance extends, however, to chemically different substances of the same group, so that we can in any individual case decide to which group a given new trypanocidal agent belongs. Thus, *e.g.*, the atoxyl-fast strain is resistant also to a number of related substances. Among hundreds of trypanocidal bodies, it is thus possible to single out those substances which contain arsenic.

It has further been shown that the resistance to trypan-red and trypan-blue is a mutual one, in spite of considerable chemical differences between these two substances, which in fact have nothing in common with one another except that they contain naphthalin rings, which are substituted in a certain position (3, 6) by sulphuric acid radicals.

Thus, we possess in this specific resistance, so to speak, a therapeutic sieve, a *cribrum therapeuticum*, with which we can undertake the classification of any new chemo-therapeutic substance. If such a substance is found to have a sterilising effect on our three different resistant strains, it necessarily belongs to a fourth chemical group. One can, of course, materially simplify this examination by employing strains that have been rendered resistant to all the groups hitherto known. Thus I have, together with Dr. Browning, obtained a strain which is resistant to atoxyl, trypan-red and fuchsin. By using such a strain, a single animal experiment suffices for the purpose of establishing any new group of chemo-therapeutic agents. For, if one injects the substance in question to such a trebly resistant mouse, the trypanosomes will either go on proliferating—then the substance must belong to one of the three known groups—or the trypanosomes are injured; in that case we have before us a new type. As you see, we are therefore enabled by means of these resistant strains to separate substances of different modes of action from one another, and thus to solve a problem which hitherto could not even be suggested. A way is thus shown us by which we may enter upon the consideration of the most intricate problems of pharmacodynamics and, if I may say so, obtain a fuller knowledge *de sedibus et causis pharmacorum*.

I also wish to lay especial stress upon my view that the drugs, also, are attracted by and bound to the protoplasm molecule by certain atom groupings. I am inclined to look upon this as somewhat analogous to the binding of the toxins and of similar proteid bodies. Yet on the other hand, there are fundamental differences between the two. For, as I have always insisted, the mode of binding the toxins is peculiar in so far as it is the result of a certain kind of assimilation which obviously consists in processes of a more or less synthetic nature. These toxin-receptors which produce immunity are bodies of a more independent character, and appear to be especially destined for the purpose of assimilation. This high degree of independence is evidenced by the fact that, in conformity with my side-chain theory, these receptors are very easily reproduced by the cell in excessive numbers, and after being separated from the cell, find their way into the blood.

I have now arrived at the opinion that in like manner a part of the chemically defined substances is attached to the cell by groups corresponding to these receptors; these atom groupings I will distinguish from the toxin-receptors by the name of "chemo-receptors." This view is more particularly supported by the fact before mentioned, that the atoxyl-fast strain is also resistant to a number of substances related to atoxyl, but otherwise showing widely different chemical characteristics. Evidently, therefore, the arsenic acid radical here represents the point of attack which is common to this series of substances. It is the arsenical radical as such, which is bound by the chemo-receptors. These chemo-receptors, must, however, be regarded as of a simpler structure than the toxin-receptors. They do not show a similar degree of independence; they cannot therefore become increased in chronic intoxications, and since they are sessile, they cannot be thrown off into the blood. The number of such chemo-receptors for poisons which a trypanosome cell possesses, represents the number of points of attack. By means of the resistant strains we can count off one by one these groups that are open to attack.

The discovery of any trypanocidal substance will always be the result of chance and experiment. Every point of attack may, of course, be liable to attack by a host of different bodies, all of which have one specific group in common. It must be the goal of our experiments to search for, by systematic chemical researches, the most suitable substances, and thus to discover the "optimum

centres." This problem is by no means an easy one, since according to my experience of many years, which agrees entirely with that of Mesnil, different animal species have different optima, and therefore the work must be specially done for each species of animals and each kind of trypanosomes. Thus, *e.g.*, in the mouse, *Trypanosoma gambiense* is far more easily attacked by arsenical preparations than the other strains of animal-pathogenic trypanosomes, whilst fuchsin and its relatives show an exactly opposite behaviour. Still, after all, this problem is not insoluble, since there are only a limited number of animal species and of trypanosomes; we may, therefore, confidently cherish the hope that the united forces of the observers who are at work on this subject in various laboratories will succeed in attaining the victory over sleeping-sickness, which is practically the most important.

Especially with a view to practical therapeutics the knowledge of the different points of attack is necessary, since it furnishes us with the possibility of fighting the disease by the combination of several chemo-therapeutic agents.

The principle of this combinatory method of therapeutics is, to attack the enemy simultaneously from two or three sides, and thus to produce a completely successful result by the combination of several different substances, each of which alone does not show a sufficient effect. Thus it has been found by Laveran and by Franke that certain infections which are influenced neither by trypan-red nor by atoxyl alone, can be cured if these two substances are used in combination. I, too, have in course of time repeatedly met with similar cases, and am convinced that substances which by themselves are not very efficient, may yet produce good results if they are used as supplements to more powerful, though not completely curative, substances. Supposing, *e.g.*, a highly trypanocidal remedy to kill ninety-eight among one hundred parasites, its administration would be followed by an instantaneous improvement, but not a definite cure, since the two surviving trypanosomes must in course of time produce a relapse. But if one combined this powerful remedy with another which, even though much weaker, yet sufficed to kill just those two surviving but attenuated parasites, then such a combination would serve to bring about the otherwise unattainable cure. In this respect a publication from the Liverpool School of Tropical Medicine is most interesting, as it demonstrates the successful result of the employment of supple-

mentary doses of perchloride of mercury in the atoxyl treatment of animals infected with sleeping-sickness.[1]

For man the combinatory treatment is of the greatest importance for a further reason. The complete sterilisation of the body, *i.e.*, the total destruction of every parasite, is often only to be accomplished by the administration of doses which come very close to the lethal dose. Such a procedure directly endangering life may be permissible in the animal experiment, but never in man. One is, however, justified in hoping that it might be possible to cure the disease without endangering life by the simultaneous administration of three, four or five substances, chosen in such a manner that their actions are concentrated on the parasites, whilst in the organism of the vertebrate host they are distributed over several different organs.

It is only natural that modern therapy which is, as a rule, brought to bear upon man under circumstances making it very difficult to judge correctly of the processes occurring during the cure, has in the last decades worked chiefly with simple bodies. On the other hand, I would remind you that in the prescriptions of the physicians of the middle of last century, a predilection for long and complex prescriptions is evident; and although nowadays this predilection may appear to us obsolete, yet it must surely have sprung from a good power of observation, enhanced by rich personal experience. Although this former practice of therapeutic procedure may have overshot the mark, it would yet appear to contain a nucleus of truth, and has certainly received the approval of science through these recent discoveries.

The importance of combined treatment is shown, lastly, in the phenomena of resistance already discussed. The fact that by frequently repeated administration of not completely sterilising doses, there is gradually acquired a resistance to the substance in question, makes it especially desirable that the main attack should be as complete as possible. This object, from what I have previously said, may probably best be carried out by a suitable combination of substances. I would like here to call your attention to the fact that two decades ago I discovered a considerable efficacy of methylene blue against certain forms of malaria. This dye has not, however, obtained any extensive employment, since its

[1] The perchloride in these cases has only the effect of an *adjuvant*, since it has been shown that its action on the trypanosomes, if administered alone, is quite inadequate.

effect is inferior to that of quinine. On the ground of our recent experience one would, I think, be perfectly justified in attempting to re-inforce the attack on the malaria parasites by employing combined doses of quinine and methylene blue.

Having now reached the end of my lecture, will you allow me once more to call your attention to the fact that the importance of these trypanosome studies is a twofold one; first, for theoretical reasons, because they afford us a deep insight into the finest mechanism of the action of the drugs; and second, for practical reasons, since by the methods of research employed and especially by the development of the combined treatment, the way has been prepared for a successful fight against trypanosome diseases. At the same time we are also justified in hoping that even beyond these narrow limits the studies may prove generally fruitful in the great campaign against infectious diseases.

ON THE TREATMENT OF TRYPANOSOMIASIS BY ATOXYL, FOLLOWED BY A MERCURIC SALT.

THE main experiments were conducted on white rats with *Trypanosoma brucei*. If animals at the height of infection were injected with atoxyl, the trypanosomes rapidly disappeared from the blood, but in 110 out of 113 cases the parasites subsequently reappeared in the blood. The authors' hypothesis is, that the administration of the drug causes the parasites to pass into "an encysted or a passive form, which is no longer attacked by the drug"; this passive form might then under altered conditions develop back into the active form and cause recurrence of the disease. It was assumed that one drug might be especially poisonous for the active, another one for the passive form. Working on these lines, they tried a number of different salts of copper, silver, lead and mercury, but found only the latter to have any effect. By treating nagana-infected rats with an injection of atoxyl at the high tide of infection, and a second dose shortly after, the parasites were removed from the blood; mercuric chloride, or Donovan's solution, was then administered, with the result that of twenty-five rats, seventeen survived, whilst only two died of trypanosomiasis, the remaining six from some other complication.

The chemical part of the paper deals with the constitution of atoxyl, which is considered to be not an anilide, but a sodium salt of an organic acid containing an amidogen group and an arsenic radical directly united to a benzene ring. All experiments should be made with freshly prepared atoxyl solutions, as older ones, especially if exposed to the light, undergo decomposition and become more poisonous to the host, and less active against the parasite.—(B. MOORE, M. NIERENSTEIN and J. L. TODD, *Bio-Chemical Journal*, 1907, No. 5/6, p. 300.)

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